Electron Transfer Reactivity of Type Zero *Pseudomonas aeruginosa* Azurin

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Supporting Information

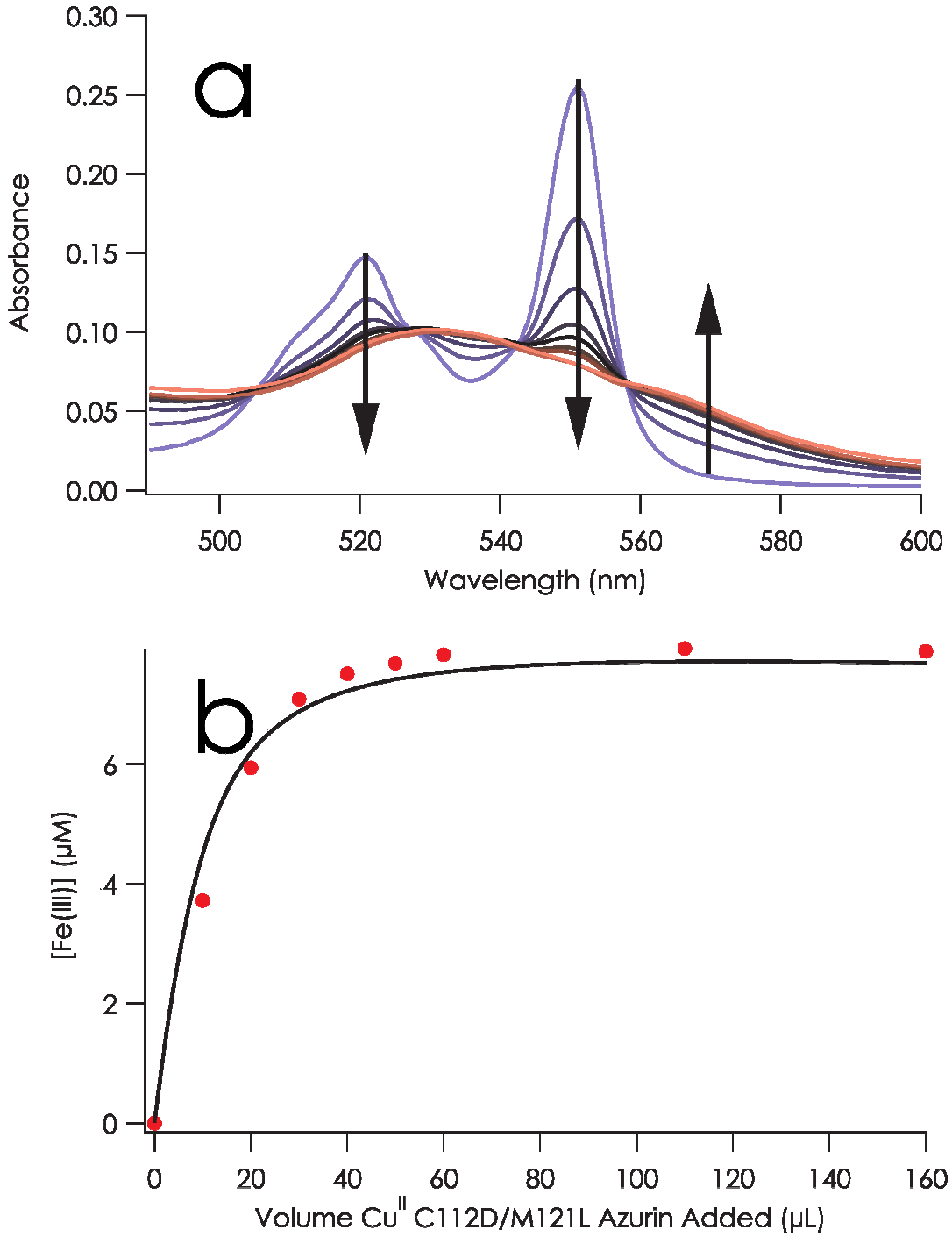
The reduction potential of C112D/M121L azurin was determined in solution by redox titration with *P. aeruginosa* cytochrome c_{551} as previously described.[1] Data were fit to the following expression:

$$[Fe^{III}] = \frac{1}{2\times(K_{eq}-1)}\times K_{eq}\left(\frac{V_{add}}{V_{add}+V_i}\times\left[Cu^{II}\right]_i+\frac{V_i}{V_{add}+V_i}\times\left[Fe^{II}\right]_i\right)- \left(K_{eq}^2\times\frac{V_{add}}{V_{add}+V_i}\times\left[Cu^{II}\right]_i+\frac{V_i}{V_{add}+V_i}\times\left[Fe^{II}\right]_i\right)^2- 4\times\left(K_{eq}-1\right)\times\left(K_{eq}\times\left(\frac{V_{add}}{V_{add}+V_i}\times\left[Cu^{II}\right]_i\times\frac{V_i}{V_{add}+V_i}\times\left[Fe^{II}\right]_i\right)^{1/2}\right) \quad \text{(S1)}$$

$[Fe^{III}]$ was determined by the expression:

$$\frac{A_{520}}{A_{551}} = 1.1427+0.55401(\%Fe^{II})-0.065012(\%Fe^{II})^2 \quad \text{(S2)}$$

which is derived from the extinction coefficients reported for cytochrome c_{551}.[2]



a) Titration of 2 mL 8.45 μM Fe^{II}-cyt *c*551 in 100 mM NaP_i pH 7.0 with Cu^{II} 1.63 mM C112D/M121L azurin. **b)** Plot of [Fe^{III}] (calculated using Eq. S2) versus [Cu^{II}] (concentrations corrected for volume) fit to Eq. (S1) K_{eq} for this titration was 2.58, giving a reduction potential of 279 mV vs NHE. Three titrations were performed, yielding a potential of 281 ± 3 mV.

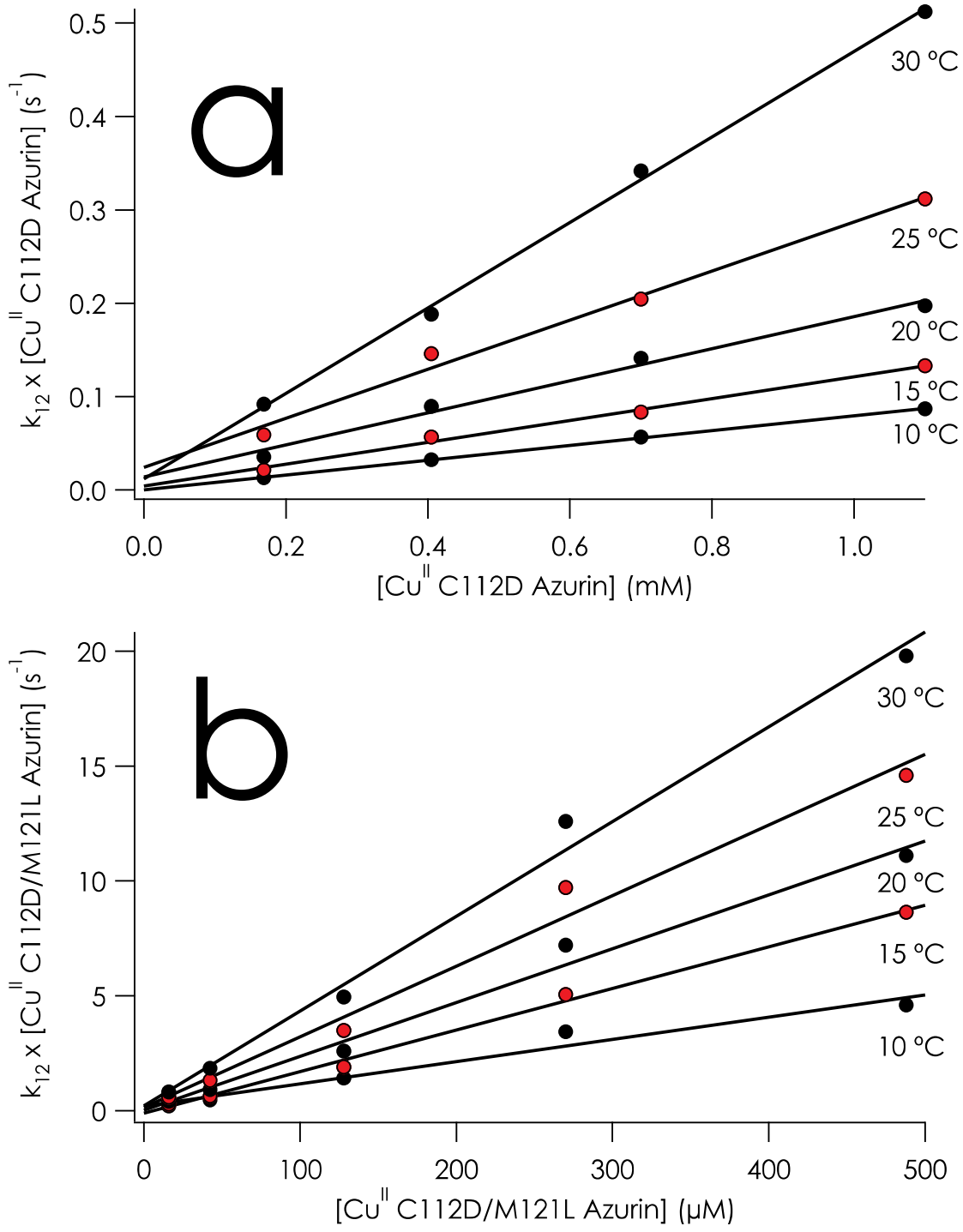


Figure S2. Concentration dependence of pseudo first order rate constants for reactions of **a)** 14 μM Cu^{I} WT azurin with Cu^{II} C112D azurin and **b)** 13.5 μM Cu^{I} WT azurin with C112D/M121L azurin in 100 mM NaPi pH 7.0 at 10, 15, 20, 25, and 30 ºC.

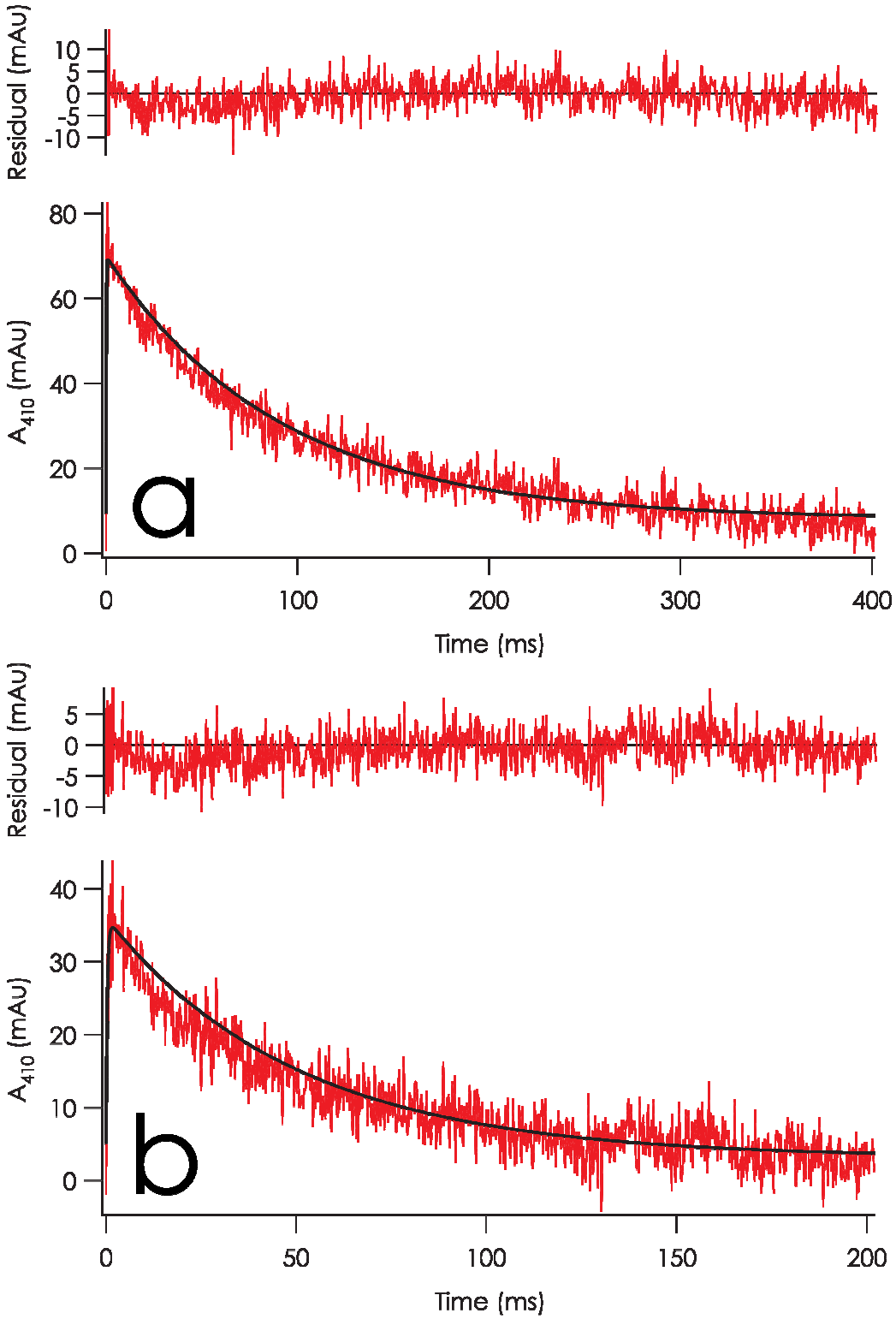


Figure S3. Transient absorption at 410 nm following 0.3 μs pulse of 10 mM NaPi/100 mM sodium formate pH 7.0 solutions of **a)** 60 μM Zn^{II} C112D azurin at 10 ºC and **b)** 50 μM Zn^{II} C112D/M121L azurin at 10 ºC. Fits corresponding to formation of $RSSR^{-}$ and its subsequent decay by dismutation ($8.2 \pm 2 \times 10^{6}$ $M^{-1}s^{-1}$) are overlaid in black.

1) Lancaster, K.M.; Sproules, S.; Palmer, J.H.; Richards, J.H.; Gray, H.B. *J. Am. Chem. Soc.* **2010**, *132*, 14590-14595.
2) Moore, G.R.; Pettigrew, G.W.; Pitt, R.C.; Williams, R.J.P. *Biochim. Biophys. Acta* **1980**, *590*, 261-271.